CLAIMS

1. A method for determining efficiency of a PCR wherein the number of copies of a particular nucleic acid sequence in a test sample is determined, comprising amplification of DNA by polymerase chain reaction of the sample itself, or a diluted stock solution of the sample itself, and one or more controlled dilutions of said sample, and registering the number of amplification cycles required to obtain a certain amount of product (CT), and estimating the efficiency of the PCR in the sample from the dependence of CT on the dilution factor.

2. A method according to claim 1, wherein the amounts of two nucleic acid sequences in a sample is compared by determining the PCR efficiencies of the two reactions according to claim 1.

3. A method according to claims 1-2, wherein the ratio of two nucleic acids in a test sample is determined using the relation:

$$\frac{N_{0A}}{N_{0B}} \propto \frac{(1+\langle E_A \rangle)^{CT_A}}{(1+\langle E_B \rangle)^{CT_B}}$$

where the CT values are measured in the test sample and the PCR efficiencies <E> are determined separately for a training set of representative samples comprising said nucleic acid sequence by the procedure in claim 1 or an equivalent procedure such as kinetic PCR.

4. A method according to claims 1-2, wherein the ratio of two nucleic acid sequences is determined in a sample using the relation:

$$\frac{N_{0A}}{N_{0B}} = K_{RS} \frac{(1+E_A)^{CT_A}}{(1+E_B)^{CT_B}}$$

also taking into account the relative sensitivity of the two PCR assays.

5. A method according to claims 1-2, wherein the amount of a nucleic acid sequence is determined in a biological sample according to either of the claims 1-4; wherein the nucleic acid is RNA, preferably one or more mRNAs that have been converted to DNA by reverse transcription or a similar process.

6. A method for diagnosing and/or classifying a disease by comparing the expression ratio of two genes by determining the ratio of the corresponding mRNAs in a sample according to either of the claims 1-5.

7. A method according to claim 6, wherein lymphoma is diagnozed by comparing the expression of at least two genes according to either of the claims 1-5, wherein the relative expression of the genes is different in clonal samples compared to healthy tissue.

8. A method according to claim 7, wherein either of the two genes is expressed in each clone of lymphocytes, and are present in a particular ratio in healthy individuals, which ratio is altered in positive samples due to clonality indicating presence of lymphoma.

9. A method according to claim 8, wherein at least a pair of the genes, the expression of which is compared, are the immunoglobulin kappa and lambda light chains.

10. A method according to claim 9, wherein the expression of the immunoglobulin kappa and lambda light chains is compared by determining the IgLκ : IgLλ mRNA ratio by reverse transcription PCR, preferably real-time PCR.

11. A method according to claim 6-10, wherein the degree of complementarity is at least 80%.

12. A method according to claim 11, wherein one or more of PCR primers are used that are complementary to
5'-TCT CGT AGT CTG CTT TGC TCA – 3' (SEQ. ID. NO.1), and
5'-CT CAT CTT TCA CCT CAC CCC – 3' (SEQ. ID. NO. 2), and
5'- C TCA GGC GTC AGG CTC – 3' (SEQ. ID. NO. 3) and
5'-C TGC ACT CAA TAA ACC CTC AAT -3' (SEQ. ID. NO. 4), respectively.

13. A method according to claim 1-6, wherein CML is diagnosed by determining the expression of bcr-abl fusion transcript.

14. A method according to claim 6, wherein the expression of three or more genes are compared.

15. A method for monitoring a disease progress, wherein the expression of two or more genes are compared.

16. A method for making disease prognosis, wherein the expression of two or more genes are compared

17. A method for comparing the presence of splicing variants of a gene by determining their relative amounts according to either of the claims 1-5.

18. A method for comparing the activities of alternative promoters by determining the relative amounts of their transcripts according to either of the claims 1-5.

19. A method for determining the amount of virus or bacteria in a sample according to either of the claims 1-5.

20. Method for diagnostic testing for cancer, including lymphoma, wherein at least the kappa:lambda expression is determined.